SUPPLEMENTARY NOTE – SYNTHETIC INFORMATION

Targeting an N-terminal Acetylation Dependent Protein Interaction

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Nature Chemical Biology: doi:10.1038/nchembio.2386
Supplementary Note Figure 1. Synthesis of NAcM-OPT (2) and NAcM-NEG (3).
**Supplementary Note Figure 2.** Synthesis of NAcM-COV (4) and NAcM-COVCTRL (16).
**Synthetic procedures:** All moisture sensitive reactions were performed using syringe-septum techniques under an atmosphere of dry N\textsubscript{2} unless otherwise noted. All glassware was dried in an oven at 140 °C for a minimum of 6 hours or flame-dried under an atmosphere of dry nitrogen prior to use. Methylene chloride and acetonitrile were dried using an aluminum oxide column. Deuterated chloroform was stored over anhydrous potassium carbonate. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F\textsubscript{254} plates, 250 μm layer thickness) and visualized by using UV lamp (254 nm) or by staining with either Vaughn\’s reagent (4.8 g of (NH\textsubscript{4})\textsubscript{6}Mo\textsubscript{7}O\textsubscript{24}\cdot4H\textsubscript{2}O and 0.2 g of Ce(SO\textsubscript{4})\textsubscript{2} in 100 mL of a 3.5 N H\textsubscript{2}SO\textsubscript{4}) or a potassium permanganate solution (1.5 g of KMnO\textsubscript{4} and 1.5 g of K\textsubscript{2}CO\textsubscript{3} in 100 mL of a 0.1% NaOH solution). Unless otherwise specified, commercially available reagents were used as received. Flash column chromatography was performed using a Biotage Isolera one and Biotage KP-SIL SNAP cartridges. Melting points were acquired on Buchi Melting Point B-545. All NMR data was collected at room temperature in CDCl\textsubscript{3} or (CD\textsubscript{3})\textsubscript{2}SO on a 400 or 500 MHz Bruker instrument. Chemical shifts (δ) are reported in parts per million (ppm) with internal CHCl\textsubscript{3} (δ 7.26 ppm for \textsuperscript{1}H and 77.00 ppm for \textsuperscript{13}C), internal DMSO (δ 2.50 ppm for \textsuperscript{1}H and 39.52 ppm for \textsuperscript{13}C), or internal TMS (δ 0.0 ppm for \textsuperscript{1}H and 0.0 ppm for \textsuperscript{13}C) as the reference. \textsuperscript{1}H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sep = septet, m= multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, qd = quartet of doublets), coupling constant(s) (J) in Hertz (Hz), and integration. Purity was assessed using LC/MS/UV/ELSD using a Waters Acquity UPLC-MS with the purity being assigned as the average determined by UV/ELSD.

1-(1-benzylpiperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)urea, NAcM-HIT (1).

m.p.: 178-180 °C. \textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 8.71 (s, 1H), 7.96 (s, 1H), 7.48 – 7.40 (m, 2H), 7.35 – 7.27 (m, 4H), 7.27 – 7.19 (m, 2H), 6.24 (d, J = 7.6 Hz, 1H), 3.56 – 3.40 (m, 3H), 2.75 – 2.65 (m, 2H), 2.07 (t, J = 11.0 Hz, 2H), 1.84 – 1.75 (m, 2H), 1.47 – 1.33 (m, 2H). \textsuperscript{13}C
NMR (101 MHz, DMSO-\textit{d}6) \( \delta \) 154.3, 141.3, 138.5, 129.7, 129.4 (q, \( J = 31.3 \) Hz), 128.8, 128.2, 126.9, 124.3 (q, \( J = 272.3 \) Hz), 121.0, 117.2 (q, \( J = 4.0 \) Hz), 113.4 (q, \( J = 4.0 \) Hz), 62.2, 51.7, 46.3, 32.0. HRMS (ESI+) \( m/z \) calcd for C\textsubscript{20}H\textsubscript{23}F\textsubscript{3}N\textsubscript{3}O \([M+H]^+\) 378.1793, found 378.1790.

N-benzyl-1-butylpiperidin-4-amine (18).

To a stirred solution of 1-butyl-4-piperidone 17 (10.0 g, 64.4 mmol, 1 equiv) and benzylamine (7.04 mL, 64.4 mmol, 1 equiv) in dry CH\textsubscript{2}Cl\textsubscript{2} (250 mL) was added acetic acid (4.43 mL, 77.0 mmol, 1.2 equiv). The resulting mixture was stirred at room temperature for 1 h and sodium triacetoxyborohydride (16.4 g, 77.0 mmol, 1.2 equiv) was added portion-wise over 30 minutes. The heterogeneous mixture was stirred at room temperature overnight (ca. 16 h). The following morning, the reaction was neutralized with a saturated aqueous solution of NaHCO\textsubscript{3}, extracted with ethyl acetate (x2), dried (MgSO\textsubscript{4}), filtered, and concentrated under reduced pressure to give 15.2 g (96% yield) of crude product 18 as a pale yellow oil. The crude product was directly carried onto the next reaction. \(^1\text{H} \) NMR (500 MHz, DMSO-\textit{d}6) \( \delta \) 7.34 – 7.26 (m, 4H), 7.19 (t, \( J = 7.1 \) Hz, 1H), 3.69 (s, 2H), 2.80 – 2.70 (m, 2H), 2.52 – 2.50 (m, 1H), 2.38 – 2.27 (m, 1H), 2.23 – 2.16 (m, 2H), 1.85 – 1.73 (m, 4H), 1.43 – 1.31 (m, 2H), 1.31 – 1.18 (m, 4H), 0.86 (t, \( J = 7.3 \) Hz, 3H). \(^{13}\text{C} \) NMR (126 MHz, DMSO-\textit{d}6) \( \delta \) 141.5, 128.0, 127.8, 126.3, 57.8, 53.8, 52.2, 49.9, 32.3, 28.9, 20.2, 14.0. LRMS (ESI+) \( m/z \) calcd for C\textsubscript{16}H\textsubscript{27}N\textsubscript{2} \([M+H]^+\) 247.4, found 247.4.

1-benzyl-1-(1-butylpiperidin-4-yl)-3-(3,4-dichlorophenyl)urea, NAcM-OPT (2).

To a stirred solution of N-benzyl-1-butylpiperidin-4-amine (18) (10.0 g, 40.6 mmol, 1 equiv) in CH\textsubscript{2}Cl\textsubscript{2} (125 mL) was added 1,2-dichloro-4-isocyanatobenzene (7.63 g, 40.6 mmol, 1 equiv) and N,N-diisopropylethylamine (10.6 mL, 60.9 mmol, 1.5 equiv). The resulting mixture was stirred at room temperature overnight (ca. 16 h) and concentrated under reduced pressure. The crude product was purified by chromatography on SiO\textsubscript{2} (MeOH:CH\textsubscript{2}Cl\textsubscript{2}, 3:97 to 7:93) to give 12 g (68% yield) of the desired product as a pure (>95%) white solid. To enrich the purity
beyond 98%, the compound was recrystallized from boiling hexanes to give 10.2 g (58% yield) of the desired product NAcM-OPT (2) as a white solid. m.p.: 118-120 °C. \(^1\)H NMR (500 MHz, Chloroform-\(d_2\)) \(\delta\) 7.42 – 7.39 (m, 3H), 7.36 – 7.29 (m, 3H), 7.21 (d, \(J = 8.7\) Hz, 1H), 6.91 (d, \(J = 8.7\) Hz, 1H), 6.27 (s, 1H), 4.49 (s, 2H), 4.44 – 4.38 (m, 1H), 2.99 (d, \(J = 11.2\) Hz, 2H), 2.31 (t, \(J = 7.8\) Hz, 2H), 2.05 (t, \(J = 11.7\) Hz, 2H), 1.85 – 1.65 (m, 4H), 1.44 (p, \(J = 7.5\) Hz, 2H), 1.30 (sext, \(J = 7.4\) Hz, 2H), 0.90 (t, \(J = 7.4\) Hz, 3H). \(^{13}\)C NMR (126 MHz, Chloroform-\(d_2\)) \(\delta\) 155.3, 138.6, 137.4, 132.4, 130.1, 129.3, 128.0, 126.1, 125.9, 121.2, 118.8, 58.4, 53.1, 52.9, 46.1, 30.1, 29.4, 20.8, 14.0. HRMS (ESI+) \(m/z\) calcd for \(C_{23}H_{30}Cl_2N_3O\) [M+H]\(^+\) 434.1766, found 434.1761.

1-benzyl-1-(1-butylpiperidin-4-yl)-3-(pyridin-3-yl)urea, NAcM-NEG (3).

To a stirred solution of N-benzyl-1-butylpiperidin-4-amine (18) (5.00 g, 20.3 mmol, 1 equiv) in \(CH_2Cl_2\) (100 mL) was added 3-isocyanatopyridine (2.44 g, 20.3 mmol, 1 equiv) and N,N-diisopropylethylamine (5.30 mL, 30.4 mmol, 1.5 equiv). The resulting mixture was stirred at room temperature overnight (ca. 16 h) and concentrated under reduced pressure. The crude product was purified by chromatography on \(SiO_2\) (MeOH:CH\(_2\)Cl\(_2\), 5:95 to 10:90) to give 5.0 g (67% yield) of the desired product NAcM-NEG (3) as a white solid. m.p.: 103-105 °C. \(^1\)H NMR (500 MHz, Chloroform-\(d_2\)) \(\delta\) 8.19 (d, \(J = 4.8\) Hz, 1H), 8.09 (s, 1H), 7.83 (d, \(J = 5.0\) Hz, 1H), 7.42 – 7.39 (m, 2H), 7.35 – 7.30 (m, 3H), 7.15 (dd, \(J = 8.6, 4.8\) Hz, 1H), 6.35 (s, 1H), 4.53 (s, 2H), 4.50 – 4.40 (m, 1H), 3.01 (d, \(J = 11.2\) Hz, 2H), 2.34 (t, \(J = 7.8\) Hz, 2H), 2.09 (t, \(J = 11.6\) Hz, 2H), 1.86 – 1.65 (m, 4H), 1.46 (p, \(J = 7.8\) Hz, 2H), 1.31 (sext, \(J = 7.3\) Hz, 2H), 0.91 (t, \(J = 7.3\) Hz, 3H). \(^{13}\)C NMR (126 MHz, Chloroform-\(d_2\)) \(\delta\) 155.6, 144.1, 141.2, 137.4, 135.8, 129.3, 128.0, 127.1, 126.1, 123.4, 58.3, 53.1, 52.8, 46.2, 30.0, 29.2, 20.8, 14.0. HRMS (ESI+) \(m/z\) calcd for \(C_{22}H_{31}N_4O\) [M+H]\(^+\) 367.2498, found 367.2497.

1-(pentan-2-yl)piperidin-4-one (20).
To a stirred solution of 4-piperidone hydrochloride (6.00 g, 44.3 mmol, 1 equiv) in dry acetonitrile (100 mL) was added potassium carbonate (30.6 g, 221 mmol, 5 equiv) and 2-iodopentane (5.58 mL, 44.3 mmol, 1 equiv). The mixture was stirred at 80 °C overnight (ca. 16 h), cooled to room temperature, filtered, diluted with ethyl acetate, washed with brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude mixture was purified by chromatography on SiO₂ (MeOH:CH₂Cl₂, 0:100 to 20:80) to give 3.35 g (48% yield) of the desired product as a yellow oil. ^1H NMR (500 MHz, Chloroform-d) δ 2.87 – 2.79 (m, 2H), 2.79 – 2.68 (m, 3H), 2.49 – 2.37 (m, 4H), 1.58 – 1.46 (m, 1H), 1.41 – 1.20 (m, 3H), 0.98 (d, J = 6.6 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H). ^13C NMR (101 MHz, Chloroform-d) δ 209.8, 58.6, 48.0, 42.0, 36.1, 20.1, 14.2, 14.1. LRMS (ESI+) m/z calcd for C₁₀H₂₀NO [M+H]+ 170.2, found 170.3.

tert-butyl (2-(((1-(pentan-2-yl)piperidin-4-yl)amino)methyl)phenyl)carbamate (22).

To a stirred solution of 1-(pentan-2-yl)piperidin-4-one (20) (2.00 g, 11.8 mmol, 1 equiv) and tert-butyl 2-(aminomethyl)phenylcarbamate (2.63 g, 11.8 mmol, 1 equiv) in CH₂Cl₂ (50 mL) was added acetic acid (0.812 mL, 14.2 mmol, 1.2 equiv). The resulting cloudy mixture was stirred at room temperature for 1 h and sodium triacetoxyborohydride (3.51 g, 16.5 mmol, 1.4 equiv) was added portion-wise over 10 min. The resulting heterogeneous mixture was stirred at room temperature overnight (ca. 16 h), quenched with a saturated aqueous solution of NaHCO₃, extracted with ethyl acetate (x2), dried (MgSO₄), filtered, and concentrated under reduced pressure to give crude tert-butyl (2-(((1-(pentan-2-yl)piperidin-4-yl)amino)methyl)phenyl)carbamate (21) as a yellow oil.

Crude 21 (4.4 g, 11.7 mmol, 1 equiv) was resuspended in CH₂Cl₂ (100 mL) and N,N-diisopropylethylamine (3.06 mL, 17.6 mmol, 1.5 equiv) and 3-(trifluoromethyl)phenyl isocyanate (1.61 mL, 11.7 mmol, 1 equiv) were added sequentially. The resulting mixture was stirred at room temperature overnight (ca. 16 h) and concentrated under reduced pressure. The crude
product was purified by chromatography on SiO₂ (MeOH:CH₂Cl₂, 2:98 to 12:88) to give 2.5 g (38% yield) of the desired product tert-butyl (2-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)phenyl)carbamate (22) as a colorless oil. ¹H NMR (500 MHz, Chloroform-d) δ 7.83 (s, 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.58 (bs, 1H), 7.38 (d, J = 7.7 Hz, 1H), 7.31 – 7.23 (m, 3H), 7.23 – 7.13 (m, 2H), 6.85 (s, 1H), 4.50 (s, 2H), 4.49 – 4.41 (m, 1H), 2.92 – 2.75 (m, 2H), 2.65 – 2.55 (m, 1H), 2.48 (t, J = 11.6 Hz, 1H), 2.34 (t, J = 11.6 Hz, 1H), 1.91 – 1.80 (m, 2H), 1.80 – 1.63 (m, 2H), 1.53 (s, 9H), 1.52 – 1.42 (m, 2H), 1.41 – 1.14 (m, 2H), 0.97 (d, J = 6.5 Hz, 3H), 0.89 (t, J = 7.0 Hz, 3H). ¹³C NMR (126 MHz, Chloroform-d) δ 155.5, 154.5, 140.4, 134.5, 132.9, 130.8 (q, J = 32.0 Hz), 128.9, 128.2, 127.1, 126.7, 125.5, 124.1 (q, J = 272.4 Hz), 122.7, 118.8 (q, J = 3.7 Hz), 116.5 (q, J = 4.0 Hz), 81.4, 59.1, 53.1, 49.7, 46.2, 42.6, 35.7, 30.4, 30.3, 28.3, 20.1, 14.2, 14.1. LRMS (ESI+) m/z calcd for C₉₀H₄₂F₃N₄O₃ [M+H]+ 563.3, found 563.5.

1-((2-aminobenzyl)-1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)urea (23).

To a stirred solution of tert-butyl (2-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)phenyl)carbamate (22) (2.50 g, 4.59 mmol, 1 equiv) in CH₂Cl₂ (50 mL) at 0 °C was slowly added trifluoroacetic acid (3.52 mL, 45.9 mmol, 10 equiv). The reaction mixture was slowly warmed to room temperature, stirred overnight (ca. 16 h), and concentrated under reduced pressure. The crude mixture was resuspended in H₂O (50 mL), neutralized with an aqueous solution of NaOH (2 M; 14 mL), extracted with CH₂Cl₂ (x3), dried (MgSO₄), filtered, and concentrated under reduced pressure to give yellow solid. The crude mixture was subjected to cursory purification by chromatography on SiO₂ (MeOH:CH₂Cl₂, 1:50 to 1:10) to give 1.8 g (88% yield) of the desired product 1-((2-aminobenzyl)-1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)urea (23) as a white solid that was immediately carried onto the next reaction. Characteristic peaks: ¹H NMR (500 MHz, Chloroform-d) δ 7.68 (s, 1H), 7.35 – 7.27 (m, 2H), 7.24 (d, J = 7.0 Hz, 1H), 7.17 – 7.08 (m, 2H), 6.82 – 6.72 (m, 3H), 1.91 – 1.80 (m, 2H), 1.80 – 1.63 (m, 2H), 1.53 (s, 9H), 1.52 – 1.42 (m, 2H), 1.41 – 1.14 (m, 2H), 0.97 (d, J = 6.5 Hz, 3H), 0.89 (t, J = 7.0 Hz, 3H).
4.68 – 4.58 (m, 1H), 4.48 (s, 2H), 4.02 (bs, 2H), 3.26 (t, J = 10.0 Hz, 2H), 3.17 – 3.03 (m, 1H),
2.86 – 2.76 (m, 2H), 2.39 (q, J = 13.0 Hz, 2H), 2.03 – 1.86 (m, 2H), 1.84 – 1.69 (m, 1H), 1.51 –
1.26 (m, 3H), 1.25 (d, J = 6.6 Hz, 3H), 0.95 (t, J = 7.1 Hz, 3H). 13C NMR (126 MHz, Chloroform-
d) δ 155.7, 144.1, 139.4, 131.1 (q, J = 32.2 Hz), 129.2, 128.7, 125.9, 124.0 (q, J = 272.3 Hz),
123.1, 121.3, 119.7 (q, J = 4.0 Hz), 119.3, 117.1, 116.8 (q, J = 4.0 Hz), 61.2, 50.7, 48.7, 47.5,
42.4, 33.3, 26.9, 26.8, 19.7, 13.8, 13.8. LRMS (ESI+) m/z calcd for C25H34F3N4O [M+H]+ 463.3,
found 463.4.

N-(2-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-
(trifluoromethyl)phenyl)ureido)methyl)phenyl)acrylamide, NAcM-COV (4).

To a stirred solution of 1-(2-aminobenzyl)-1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-
(trifluoromethyl)phenyl)urea (23) (1.70 g, 3.68 mmol, 1 equiv) and N,N-diisopropylethylamine
(0.813 mL, 4.67 mmol, 1.2 equiv) in CH2Cl2 (20 mL) was added dropwise freshly distilled
acryloyl chloride (0.355 mL, 4.67 mmol, 1.2 equiv). The reaction mixture was slowly warmed to
room temperature, stirred for 3 h, diluted with CH2Cl2, washed with a saturated aqueous solution
of NaHCO3, brine, dried (MgSO4), filtered, and concentrated under reduced pressure. The crude
mixture was purified by chromatography on SiO2 (MeOH:CH2Cl2, 3:97 to 12:88) to give 0.780 g
(39% yield) of a racemic mixture of the desired product NAcM-COV (4) as a white solid. m.p.:
95-97 °C. 1H NMR (500 MHz, Chloroform-d) δ 8.66 (bs, 1H), 7.93 (s, 1H), 7.57 – 7.51 (m, 3H),
7.36 (d, J = 7.7 Hz, 1H), 7.32 – 7.27 (m, 2H), 7.25 – 7.16 (m, 2H), 6.54 – 6.31 (m, 2H), 5.77 (d,
J = 9.6 Hz, 1H), 4.52 (s, 2H), 4.25 (bs, 1H), 2.88 (d, J = 11.1 Hz, 2H), 2.64 (q, J = 6.7 Hz, 1H),
2.47 (t, J = 11.6 Hz, 1H), 2.40 – 2.26 (m, 1H), 1.95 – 1.72 (m, 4H), 1.55 – 1.49 (m, 1H), 1.44 –
1.17 (m, 3H), 0.99 (d, J = 6.5 Hz, 3H), 0.90 (t, J = 10.0 Hz, 3H). 13C NMR (126 MHz,
Chloroform-d) δ 164.8, 155.7, 140.0, 134.7, 132.1, 130.9 (q, J = 32.2 Hz), 130.4, 129.0, 128.5,
128.4, 127.9, 126.7, 125.3, 124.1 (q, J = 272.3 Hz), 123.1, 119.3 (q, J = 4.0 Hz), 116.9 (q, J =
N-(2-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)phenyl)propionamide NAcM-COVCTRL (16).

To a stirred solution of 1-(2-aminobenzyl)-1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)urea (23) (0.180 g, 0.389 mmol, 1 equiv) and N,N-diisopropylethylamine (0.0813 mL, 0.467 mmol, 1.2 equiv) in CH$_2$Cl$_2$ (2 mL) was added dropwise freshly distilled propionyl chloride (0.0340 mL, 0.467 mmol, 1.2 equiv). The reaction mixture was slowly warmed to room temperature, stirred for 3 h, diluted with CH$_2$Cl$_2$, washed with a saturated aqueous solution of NaHCO$_3$, brine, dried (MgSO$_4$), filtered, and concentrated under reduced pressure. The crude mixture was purified by chromatography on SiO$_2$ (MeOH:CH$_2$Cl$_2$, 3:97 to 12:88) to give 0.100 g (50% yield) of a racemic mixture of the desired product NAcM-COVCTRL (16) as a white solid. m.p.: 90-92 °C. $^1$H NMR (500 MHz, Chloroform-d) δ 8.12 (bs, 1H), 7.94 (s, 1H), 7.61 – 7.47 (m, 2H), 7.42 – 7.34 (m, 2H), 7.33 – 7.24 (m, 2H), 7.22 – 7.17 (m, 2H), 4.48 (s, 2H), 4.35 (bs, 1H), 2.88 (d, $J = 11.1$ Hz, 2H), 2.64 (q, $J = 6.6$ Hz, 1H), 2.51 – 2.42 (m, 3H), 2.40 – 2.26 (m, 1H), 1.91 – 1.70 (m, 4H), 1.59 – 1.44 (m, 1H), 1.44 – 1.28 (m, 3H), 1.26 (t, $J = 7.7$ Hz, 3H), 0.99 (d, $J = 6.5$ Hz, 3H), 0.90 (t, $J = 7.0$ Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-d) δ 173.5, 155.5, 140.1, 134.6, 132.6, 130.9 (q, $J = 32.0$ Hz), 128.9, 128.4, 127.5, 126.8, 125.6, 124.1 (q, $J = 272.4$ Hz), 122.9, 119.1 (q, $J = 4.0$ Hz), 116.7 (q, $J = 4.0$ Hz), 59.2, 53.2, 49.6, 46.4, 43.1, 35.5, 30.2, 30.0, 29.9, 20.1, 14.2, 14.1, 9.8. HRMS (ESI+) m/z calcd for C$_{28}$H$_{36}$F$_3$N$_4$O$_2$ [M+H]$^+$ 519.2946, found 519.2941.
(a) $^1$H NMR and (b) $^{13}$C NMR for NAcM-HIT

**Figure:**

- (a) $^1$H NMR spectrum showing peaks at various ppm values.
- (b) $^{13}$C NMR spectrum with labeled peaks at specific ppm values.

**Chemical Structures:**

- NAcM-HIT (1) with structural formulas corresponding to the NMR spectra.
UPLC-MS (UV/ELSD) characterization of NAcM-HIT
(a) $^1$H NMR and (b) $^{13}$C NMR for NAcM-OPT
(a) $^1$H NMR and (b) $^{13}$C NMR for NAcM-NEG

NAcM-NEG (3)
UPLC-MS (UV/ELSD) characterization of NAcM-NEG
(a) $^1$H NMR and (b) $^{13}$C NMR for NAcM-COV
UPLC-MS (UV/ELSD) characterization of NAcM-COV
Characterization of compounds from Supplementary Table 4

2-chloro-N-(2-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)phenyl)acetamide (5). Average Purity (ELSD/UV): 90%. $^1$H NMR (500 MHz, Chloroform-d) $\delta$ 9.17 (s, 1H), 7.83 (s, 1H), 7.59 (bs, 1H), 7.42 (d, $J = 8.1$ Hz, 1H), 7.29 – 7.01 (m, 6H), 4.59 – 4.52 (m, 1H), 4.43 (s, 2H), 4.20 (s, 2H), 3.03 (d, $J = 11.5$ Hz, 2H), 2.86 (bs, 1H), 2.74 – 2.37 (m, 2H), 2.15 – 1.93 (m, 2H), 1.89 – 1.68 (m, 2H), 1.68 – 1.60 (m, 1H), 1.36 – 1.16 (m, 3H), 1.08 (d, $J = 6.0$ Hz, 3H), 0.85 (t, $J = 7.0$ Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-d) $\delta$ 166.3, 155.5, 139.9, 133.4, 130.8 (q, $J = 32.0$ Hz), 129.0, 128.4, 127.8, 126.7, 124.3, 124.0 (q, $J = 273.3$ Hz), 123.1, 121.1, 119.4 (q, $J = 3.9$ Hz), 116.8 (q, $J = 4.0$ Hz), 60.8, 51.3, 49.2, 47.1, 42.9, 42.3, 34.0, 28.2, 28.1, 19.8, 13.9, 13.8. LRMS (APCI+) m/z calcd for C$_{27}$H$_{34}$ClF$_3$N$_4$O$_2$ [M+H]$^+$ 539.2, found 539.4.

N-(3-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)phenyl)acrylamide (6). Average Purity (ELSD/UV): 99%. $^1$H NMR (500 MHz, Chloroform-d) $\delta$ 9.22 (s, 1H), 7.67 (s, 1H), 7.64 (d, $J = 8.0$ Hz, 1H), 7.30 – 7.20 (m, 1H), 7.20 (t, $J = 7.8$ Hz, 1H) 7.17 – 7.06 (m, 2H), 6.88 (d, $J = 7.6$ Hz, 1H), 6.42 (dd, $J = 16.9$, 10.1 Hz, 1H), 6.30 (d, $J = 16.8$ Hz, 1H), 5.60 (d, $J = 10.0$ Hz, 1H), 4.42 – 4.37 (m, 1H), 4.34 (s, 2H), 2.96 – 2.86 (m, 2H), 2.80 (bs, 1H), 2.60 – 2.45 (m, 2H), 2.08 – 1.92 (m, 2H), 1.86 – 1.67 (m, 2H), 1.65 – 1.58 (m, 1H), 1.32 – 1.23 (m, 1H), 1.23 – 1.07 (m, 2H), 1.04 (d, $J = 6.5$ Hz, 3H), 0.82 (t, $J = 6.9$ Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-d) $\delta$ 164.3, 155.6, 139.7, 139.2, 138.6, 131.3, 130.8 (q, $J = 32.1$ Hz), 129.6, 129.1, 127.6, 126.1 (q, $J = 272.5$ Hz), 123.2, 121.8, 119.5, 119.4 (q, $J = 4.0$ Hz), 117.9, 116.8 (d, $J = 4.0$ Hz), 60.9, 51.4, 48.8, 47.1, 45.8, 33.6, 28.0, 19.6, 13.8, 13.7. LRMS (APCI+) m/z calcd for C$_{28}$H$_{36}$F$_3$N$_4$O$_2$ [M+H]$^+$ 517.3, found 517.5.
2-chloro-N-(3-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)phenyl)acetamide (7). Average Purity (ELSD/UV): 99%. $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 8.87 (s, 1H), 7.63 (s, 1H), 7.50 (s, 1H), 7.42 (d, $J = 8.0$ Hz, 1H), 7.30 (d, $J = 8.0$ Hz, 1H), 7.27 – 7.18 (m, 2H), 7.14 (d, $J = 7.7$ Hz, 1H), 6.99 (d, $J = 7.7$ Hz, 1H), 6.93 (bs, 1H), 4.42 (s, 2H), 4.38 – 4.35 (m, 1H), 4.09 (s, 2H), 2.97 (d, $J = 11.2$ Hz, 2H), 2.87 – 2.71 (m, 1H), 2.67 – 2.46 (m, 2H), 2.13 – 1.86 (m, 2H), 1.86 – 1.74 (m, 2H), 1.69 – 1.54 (m, 1H), 1.41 – 1.25 (m, 1H), 1.24 – 1.13 (m, 2H), 1.05 (d, $J = 6.6$ Hz, 3H), 0.83 (t, $J = 6.9$ Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-$d$) $\delta$ 164.6, 155.6, 139.6, 139.0, 138.0, 130.9 (q, $J = 32.2$ Hz), 129.8, 129.1, 123.7 (q, $J = 272.5$ Hz), 123.1, 122.6, 119.6, 119.5 (q, $J = 4.0$ Hz), 118.0, 116.7 (d, $J = 4.0$ Hz), 60.6, 51.8, 49.1, 47.0, 45.9, 43.1, 34.1, 28.5, 19.8, 13.9, 13.8. LRMS (APCI+) $m/z$ calcd for C$_{27}$H$_{35}$ClF$_3$N$_4$O$_2$ [M+H]$^+$ 539.2, found 539.4.

N-(2-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)benzyl)acrylamide (8). Average Purity (ELSD/UV): 99%. $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ $^1$H NMR $\delta$ 7.83 (s, 1H), 7.75 (s, 1H), 7.58 – 7.45 (m, 2H), 7.35 (d, $J = 6.8$ Hz, 1H), 7.30 – 7.15 (m, 5H), 6.40 – 6.15 (m, 2H), 5.58 (d, $J = 9.2$ Hz, 1H), 4.94 (s, 2H), 4.47 (t, $J = 11.8$ Hz, 1H), 4.35 (d, $J = 5.8$ Hz, 2H), 3.20 – 3.10 (m, 2H), 2.96 (s, 1H), 2.86 – 2.56 (m, 2H), 2.34 – 2.05 (m, 2H), 1.93 (d, $J = 13.3$ Hz, 2H), 1.76 – 1.66 (m, 1H), 1.50 – 1.22 (m, 3H), 1.17 (d, $J = 6.6$ Hz, 3H), 0.92 (t, $J = 7.0$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ $^{13}$C NMR $\delta$ 166.2, 155.6, 139.9, 135.4, 135.0, 130.6 (q, $J = 32.2$ Hz), 130.5, 130.2, 128.8, 127.8, 127.6, 126.6, 124.7, 124.0 (q, $J = 272.5$ Hz), 123.4, 119.2 (q, $J = 4.0$ Hz), 117.0 (d, $J = 4.0$ Hz), 60.7, 50.8, 48.9, 47.2, 42.9, 39.5, 33.7, 27.7, 19.6, 13.8, 13.7. LRMS (APCI+) $m/z$ calcd for C$_{29}$H$_{36}$F$_3$N$_4$O$_2$ [M+H]$^+$ 531.3, found 531.3.

2-chloro-N-(2-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)benzyl)acetamide (9). Average Purity (ELSD/UV): 99%. $^1$H
NMR (500 MHz, Chloroform-d) δ 7.87 (s, 1H), 7.72 (s, 1H), 7.54 (d, J = 8.3 Hz, 1H), 7.39 – 7.31 (m, 1H), 7.32 – 7.22 (m, 4H), 7.21 (d, J = 6.0 Hz, 2H), 4.87 (s, 2H), 4.74 – 4.69 (m, 1H), 4.40 (d, J = 5.8 Hz, 2H), 4.05 (s, 2H), 3.18 – 3.15 (m, 2H), 3.05 – 2.95 (m, 1H), 2.77 – 2.68 (m, 2H), 2.20 – 2.14 (m, 2H), 1.93 (d, J = 13.5 Hz, 2H), 1.75 – 1.63 (m, 1H), 1.52 – 1.22 (m, 3H), 1.18 (d, J = 6.6 Hz, 3H), 0.93 (t, J = 7.0 Hz, 3H).

13C NMR (126 MHz, Chloroform-d) δ 167.0, 155.6, 139.8, 135.2, 134.7, 130.8 (q, J = 32.1 Hz), 130.0, 129.0, 128.2, 127.9, 124.9, 124.1 (q, J = 272.4 Hz), 123.2, 119.4 (q, J = 3.9 Hz), 117.0 (q, J = 4.0 Hz), 60.8, 50.9, 48.9, 47.3, 43.0, 42.5, 40.0, 33.8, 27.7, 19.7, 13.9, 13.8. LRMS (APCI+) m/z calcd for C28H37ClF3N4O2 [M+H]+ 553.3, found 553.4.

N-(3-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)benzyl)acrylamide (10). Average Purity (ELSD/UV): 99%.

1H NMR (500 MHz, Chloroform-d) δ 7.67 (s, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.30 – 7.25 (m, 2H), 7.23 – 7.14 (m, 4H), 6.92 (bs, 1H), 6.77 (bs, 1H), 6.31 – 6.12 (m, 2H), 5.60 (d, J = 9.9 Hz, 1H), 4.48 (s, 2H), 4.42 (d, J = 6.1 Hz, 2H), 4.42 – 4.40 (m, 1H), 2.95 (d, J = 11.3 Hz, 2H), 2.75 (bs, 1H), 2.55 (t, J = 11.8 Hz, 1H), 2.46 (t, J = 11.6 Hz, 1H), 1.96 – 1.68 (m, 4H), 1.65 – 1.53 (m, 1H), 1.43 – 1.30 (m, 1H), 1.30 – 1.20 (m, 2H), 1.05 (d, J = 6.6 Hz, 3H), 0.91 (t, J = 6.9 Hz, 3H).

13C NMR (126 MHz, Chloroform-d) δ 165.7, 155.5, 139.7, 139.3, 138.3, 130.9 (q, J = 32.2 Hz), 130.6, 129.3, 129.1, 127.1, 126.7, 125.4, 124.9, 123.9 (q, J = 272.6 Hz), 122.9, 119.4 (q, J = 3.9 Hz), 116.6 (q, J = 3.9 Hz), 59.8, 52.5, 49.2, 46.6, 45.8, 43.2, 34.7, 29.2, 19.9, 14.0, 13.9. LRMS (APCI+) m/z calcd for C29H38F3N4O2 [M+H]+ 531.3, found 531.4.

2-chloro-N-(3-((1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)methyl)benzyl)acetamide (11). Average Purity (ELSD/UV): 99%.

1H NMR (500 MHz, Chloroform-d) δ 7.71 (s, 1H), 7.39 – 7.26 (m, 4H), 7.26 – 7.17 (m, 4H), 6.97 (bs, 1H), 4.57 (s, 2H), 4.57 – 4.49 (m, 1H), 4.44 (d, J = 5.9 Hz, 2H), 4.06 (s, 2H), 3.20 – 3.05 (m, 2H), 2.93 (bs, 1H), 2.79 – 2.56 (m, 2H), 2.35 – 2.10 (m, 2H), 1.84 (d, J = 13.3 Hz, 2H), 1.75 (bs, 1H).
\[ \text{H}, 1.53 - 1.22 \text{ (m, 3H)}, 1.18 \text{ (d, } J = 6.5 \text{ Hz, 3H)}, 0.93 \text{ (t, } J = 6.9 \text{ Hz, 3H)}. \]  
\[ ^{13}\text{C NMR (126 MHz, CDCl}_3) \delta 166.3, 155.5, 139.6, 138.7, 138.3, 131.0 \text{ (q, } J = 32.0 \text{ Hz)}, 129.6, 129.2, 127.2, 125.7, \]
\[ 125.4, 124.0 \text{ (q, } J = 272.6 \text{ Hz)}, 123.1, 119.6 \text{ (q, } J = 4.0 \text{ Hz)}, 116.7 \text{ (q, } J = 4.0 \text{ Hz)}, 60.8, 51.6, \]
\[ 49.0, 47.2, 46.0, 43.5, 42.7, 33.9, 28.2, 19.8, 13.9, 13.8 \text{. LRMS (APCI+) } m/z \text{ calcd for } C_{28}H_{37}ClF_3N_4O_2 [M+H]^+ 553.3, \text{ found 553.4.} \]

\[ N-(2-(1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)ethyl)acrylamide (12). \]
\[ \text{Average Purity (ELSD/UV): 99\%. } ^{1}\text{H NMR (500 MHz, Chloroform-}d) \delta 9.91 \text{ (bs, 1H)}, 8.22 \text{ (s, 1H)}, 7.91 \text{ (d, } J = 8.1 \text{ Hz, 1H)}, 7.39 \text{ (t, } J = 8.0 \text{ Hz, 1H)}, 7.25 \text{ (d, } J = 7.8 \text{ Hz, 1H)}, 6.98 \text{ (bs, 1H)}, \]
\[ 6.40 \text{ (d, } J = 17.0 \text{ Hz, 1H)}, 6.19 \text{ (dd, } J = 16.9, 10.3 \text{ Hz, 1H)}, 5.72 \text{ (d, } J = 10.3 \text{ Hz, 1H)}, 4.41 \text{ (t, } J = 12.1 \text{ Hz, 1H)}, \]
\[ 3.38 \text{ (s, 4H)}, 3.07 \text{ (d, } J = 11.4 \text{ Hz, 2H)}, 2.85 \text{ (bs, 1H)}, 2.64 \text{ (t, } J = 11.6 \text{ Hz, 1H)}, \]
\[ 2.55 \text{ (t, } J = 11.8 \text{ Hz, 1H)}, 2.04 - 1.87 \text{ (m, 2H)}, 1.79 - 1.72 \text{ (m, 2H)}, 1.71 - 1.60 \text{ (m, 1H)}, 1.45 - \]
\[ 1.22 \text{ (m, 3H)}, 1.12 \text{ (d, } J = 6.6 \text{ Hz, 3H)}, 0.94 \text{ (t, } J = 6.9 \text{ Hz, 3H}). \]  
\[ ^{13}\text{C NMR (126 MHz, Chloroform-}d) \delta 167.5, 155.5, 140.9, 130.7 \text{ (q, } J = 32.0 \text{ Hz)}, 129.6, 129.0, 127.8, 124.3 \text{ (q, } J = 272.3 \text{ Hz),} \]
\[ 122.8, 118.8 \text{ (q, } J = 3.9 \text{ Hz)}, 116.5 \text{ (q, } J = 4.0 \text{ Hz)}, 60.2, 50.9, 49.3, 46.9, 40.9, 40.1, 34.6, 29.3, \]
\[ 29.2, 19.9, 14.0, 13.9 \text{. LRMS (APCI+) } m/z \text{ calcd for } C_{23}H_{34}ClF_3N_4O_2 [M+H]^+ 455.3, \text{ found 455.2.} \]

\[ 2\text{-chloro-N-(2-(1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)ethyl)acetamide (13). Average Purity (ELSD/UV): 95\%. } ^{1}\text{H NMR (500 MHz, Acetone-}d_6) \delta 9.12 \text{ (s, 1H)}, 8.40 \text{ (bs, 1H)}, 8.34 \text{ (s, 1H)}, 7.97 \text{ (d, } J = 8.3 \text{ Hz, 1H)}, \]
\[ 7.48 \text{ (t, } J = 8.0 \text{ Hz, 1H)}, 7.29 \text{ (d, } J = 7.8 \text{ Hz, 1H)}, 4.49 - 4.30 \text{ (m, 1H)}, 4.26 \text{ (s, 2H)}, 3.44 \text{ (m, 4H)}, 3.25 \]
\[ - 3.10 \text{ (m, 2H)}, 3.03 \text{ (bs, 1H)}, 2.95 - 2.68 \text{ (m, 2H)}, 2.12 - 1.91 \text{ (m, 4H)}, 1.87 - 1.57 \text{ (m, 2H)}, \]
\[ 1.40 \text{ (m, 2H)}, 1.18 \text{ (d, } J = 6.4 \text{ Hz, 3H)}, 0.94 \text{ (t, } J = 6.9 \text{ Hz, 3H}). \]  
\[ ^{13}\text{C NMR (126 MHz, Acetone-}d_6) \delta 168.8, 154.8, 142.0, 130.1 \text{ (q, } J = 31.7 \text{ Hz)}, 129.2, 124.6 \text{ (q, } J = 271.5 \text{ Hz)}, 122.5, 117.9 \text{ (q, } J = 4.0 \text{ Hz)}, 115.5 \text{ (q, } J = 4.1 \text{ Hz)}, 59.9, 50.9, 49.3, 46.3, 42.1, 40.7, 40.0, 34.2, 19.5, 13.4, 13.0. \]
\[ \text{LRMS (APCI+) } m/z \text{ calcd for } C_{22}H_{30}ClF_3N_4O_2 [M+H]^+ 477.2, \text{ found 477.2.} \]
N-(3-(1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)propyl)acrylamide (14).

Average Purity (ELSD/UV): 98%. $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.92 (s, 1H), 7.82 (bs, 1H), 7.42 – 7.31 (m, 2H), 7.26 (d, $J = 7.8$ Hz, 1H), 6.45 – 6.17 (m, 2H), 5.63 (d, $J = 9.6$ Hz, 1H), 4.55 – 4.35 (m, 1H), 3.47 (t, $J = 8.0$ Hz, 2H), 3.37 (q, $J = 5.8$ Hz, 2H), 3.30 – 3.21 (m, 2H), 3.09 (bs, 1H), 2.87 – 2.75 (m, 2H), 2.56 – 2.36 (m, 2H), 2.04 – 1.69 (m, 4H), 1.58 – 1.15 (m, 7H), 0.97 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-$d$) $\delta$ 166.8, 155.3, 140.2, 130.8 (q, $J = 32.4$ Hz) 130.8, 129.1, 126.6, 124.0 (q, $J = 272.3$ Hz), 123.4, 119.3 (q, $J = 3.9$ Hz), 117.1 (q, $J = 4.0$ Hz), 61.3, 50.5, 49.0, 47.7, 40.2, 37.4, 33.3, 30.1, 27.4, 19.6, 13.8, 13.7. LRMS (APCI+) m/z calcd for C$_{24}$H$_{36}$F$_3$N$_4$O$_2$ [M+H]$^+$ 469.3, found 469.3.

2-chloro-N-(3-(1-(1-(pentan-2-yl)piperidin-4-yl)-3-(3-(trifluoromethyl)phenyl)ureido)propyl)acetamide (15). Average Purity (ELSD/UV): 95%. $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.85 (s, 1H), 7.67 (d, $J = 8.3$ Hz, 1H), 7.53 (bs, 1H), 7.44 – 7.31 (m, 2H), 7.28 (d, $J = 6.9$ Hz, 1H), 4.23 (bs, 1H), 4.08 (s, 2H), 3.41 (t, $J = 7.9$ Hz, 2H), 3.35 (q, $J = 6.1$ Hz, 2H), 3.20 – 3.10 (m, 2H), 2.95 (bs, 1H), 2.74 – 2.54 (m, 2H), 2.25 – 2.10 (m, 2H), 2.00 – 1.77 (m, 4H), 1.75 – 1.60 (m, 1H), 1.50 – 1.26 (m, 3H), 1.17 (s, 3H), 0.95 (t, $J = 6.9$ Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-$d$) $\delta$ 167.3, 155.1, 139.9, 131.0 (q, $J = 32.1$ Hz), 129.2, 123.8 (q, $J = 268.5$ Hz), 123.2, 119.4 (q, $J = 3.9$ Hz), 116.9 (q, $J = 3.9$ Hz), 60.5, 51.9, 49.2, 47.1, 42.6, 40.1, 37.6, 34.1, 30.3, 28.6, 19.8, 13.9, 13.8. LRMS (APCI+) m/z calcd for C$_{23}$H$_{35}$ClF$_3$N$_4$O$_2$ [M+H]$^+$ 491.2, found 491.1.